DIFFERENTIAL RECOGNITION OF CHLOROPLAST AND CYTOPLASMIC MESSENGER RNA BY 70S AND 80S RIBOSOMAL SYSTEMS

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1. Introduction

Chloroplasts are known to contain DNA and to be competent to express the genetic information contained in that DNA. They have their own DNA-dependent RNA polymerase and also their own protein-synthesizing apparatus [1]. The latter is a 70S ribosomal system, similar in many respects to that of prokaryotes but quite distinct from the 80S ribosomal system of the plant cytoplasm. In this report we show that it is possible to differentiate between the messenger RNA in the chloroplast and that present in the cytoplasm. Chloroplast mRNA can be translated by a 70S ribosomal, protein-synthesizing system from *E. coli* (*E. coli* S30) but not by an 80S ribosomal system from wheat germ (wheat germ S30), whereas mRNA in the cytoplasm can be translated by a wheat germ S30 but not by an *E. coli* S30.

2. Materials and methods

S30 fractions were prepared from cells of *E. coli* MRE600 by the method of Modellel [2]. Wheat germ extracts were obtained from isolated embryos [3] as described by Roberts and Paterson [4].

Spinach chloroplasts were isolated as described by Bottomley et al. [5] and the RNA was extracted by a phenol-SDS method [6]. Leaf cytoplasmic RNA was extracted from the 10 000 *g* supernatant following removal of chloroplasts. Total leaf RNA and root RNA from spinach were isolated by grinding the tissue in liquid N_2 and extracting RNA by the phenol-SDS method. RNA preparations were not fractionated or enriched for mRNA so the bulk of the RNA added to the protein synthesizing systems was ribosomal and transfer RNA.

2.1. *Measurement of RNA directed protein synthesis*

2.1.1. Wheat germ

Assays were carried out in a final volume of 50 μl essentially as described by Roberts and Paterson [4]. Each assay mixture contained 10 μl of preincubated wheat germ S30, 20 mM HEPES (pH 7.6), 2 mM dithiothreitol, 1 mM ATP, 0.1 mM GTP, 8 mM creatine phosphate, 40 μg/ml creatine phosphokinase, 105 mM KCl, the magnesium acetate concentration was optimized for each RNA (usually about 2 mM) and 1 μCi [^{35}S]methionine (Amersham, 240 Ci/mmole). After 70 min at 25°C the reaction was stopped by cooling and the addition of unlabelled methionine. Aliquots of 10 μl were removed and precipitated in the presence of carrier bovine serum albumin and unlabelled methionine by 5% TCA at 80°C for 15 min. The precipitates were transferred to Whatman GFC discs and washed with 5% TCA, ethanol and diethyl ether. They were then dried and radioactivity was determined in a Packard liquid scintillation spectrometer as previously described [5].

2.1.2. *E. coli*

Protein synthesis using the *E. coli* S30 was carried out by a modification of the method of Zubay [7]. The 50 μl assay volumes contained 30 mM Tris-acetate pH 8.2, 40 mM potassium acetate, 20 mM ammonium acetate, 2 mM ATP, 0.4 mM GTP, 7 mM phosphoenol pyruvate, 1 mM dithiothreitol, 1 μg pyruvate kinase, 2.5 mM each of 19 amino acids and 1–5 μCi [^{35}S]-methionine. Aliquots were removed after 20 min

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at 37°C and processed as described for the wheat germ system.

2.1.3. SDS gel electrophoresis

After incubation the reaction mixture was diluted with cold methionine, 1 volume of 10% TCA was added and the mixture heated at 80°C for 5 min. The precipitate was collected by centrifugation, washed with 5% TCA, ethanol and then acetone and dissolved in an SDS buffer as described by Laemmli and Favre [8]. Polypeptides were separated as described by Laemmli and Favre [8] and autoradiographs made as described previously [5].

3. Results

When chloroplast RNA was added to a wheat germ S30 little stimulation of protein synthesis was obtained (fig.1a). Moreover, increasing the amount of chloroplast RNA above 100 μg/ml actually decreased the response in the wheat germ system. However, when spinach chloroplast RNA was added to an *E. coli* S30 a marked stimulation of amino acid incorporation was observed (fig.1b).

The converse of this differential response pattern was obtained when root RNA (a convenient source of cytoplasmic RNA uncontaminated with chloroplast RNA) was added to the *E. coli* and wheat germ S30. Root RNA stimulated protein synthesis when added to the wheat germ cell-free system but gave only poor stimulation when added to the *E. coli* system (fig.1a,1b).

Addition of total leaf RNA from spinach caused a stimulation of amino acid incorporation in both S30 systems (fig.1a,1b), a result to be expected since both chloroplast and cytoplasmic RNAs are present in such a preparation. Leaf cytoplasmic RNA not only programmed the 80S system as efficiently as root RNA but also gave some stimulation with the 70S system due to the leaf cytoplasmic RNA preparations containing significant amounts of chloroplast RNA (data not shown).

3.1. *Analysis of products*

The radioactive products of the reactions were electrophoresed under dissociating conditions on SDS-polyacrylamide gels and the radioactive bands located by autoradiography. The pattern of products obtained from the chloroplast RNA-directed protein synthesis in the *E. coli* S30 (fig.2b) demonstrated

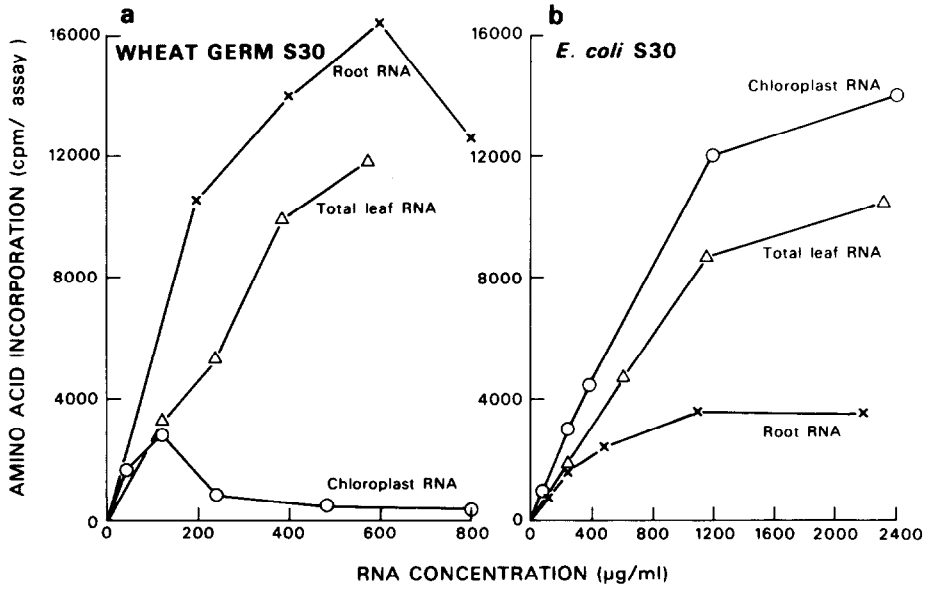

Fig.1. (a) Incorporation of radioactive amino acid into the hot TCA insoluble fraction by the wheat germ cell-free protein synthesizing system. (o——o) Chloroplast RNA; (X——X) root RNA; (△——△) total leaf RNA. (b) Incorporation of radioactive amino acids into the hot TCA insoluble fraction by the *E. coli* cell- free synthesizing system.

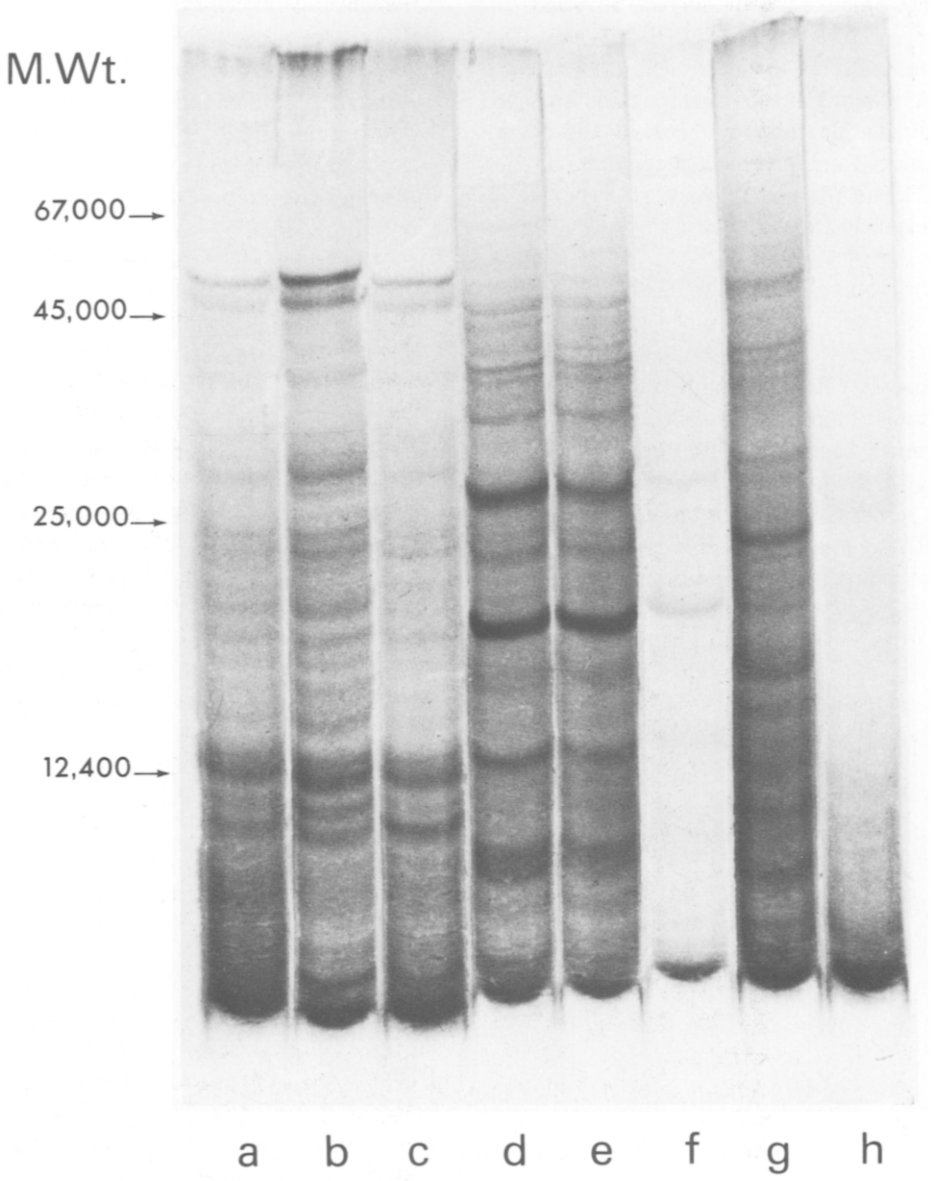

Fig.2. Autoradiographs of SDS-gel electrophoresis of the products from incorporation of [^{35}S]methionine by the wheat germ and the *E. coli* cell-free protein synthesizing system. (a) *E. coli* S30 with leaf cytoplasmic RNA. (b) *E. coli* S30 with chloroplast RNA. (c) *E. coli* S30 with total leaf RNA. (d) Wheat germ S30 with total leaf RNA. (e) Wheat germ S30 with leaf cytoplasmic RNA. (f) Wheat germ S30 with chloroplast RNA. (g) Wheat germ S30 with root RNA. (h) *E. coli* S30 with root RNA. Approximately equal amounts of TCA precipitable counts were loaded onto the gels except in the case of 2f and 2h.

that discrete polypeptides, ranging in size from 5000 to 52 000 daltons, were being synthesized. The major high mol. wt. band of radioactivity comigrated with the large subunit of ribulose diphosphate carboxylase of mol. wt. 52 000. Hartley et al. [9] recently reported that this protein subunit is synthesized in an *E. coli* S30 programmed with chloroplast RNA. It seems likely that the *E. coli* S30 system described is translating chloroplast messenger RNA with fidelity.

Although the response of the wheat germ S30 to the addition of chloroplast RNA was quantitatively small the possibility existed that this system was capable of translating chloroplast messenger RNA, albeit very inefficiently. When the reaction was scaled up so that enough radioactive product was obtained to permit electrophoretic analysis, a number of faint but discrete bands could be detected on the autoradiograph (fig.2f). The pattern of these bands, however, was quite different from that obtained with the *E. coli* system. In fact it showed a close resemblance to the pattern obtained from a wheat germ S30 programmed with cytoplasmic RNA (see later). It seems likely that the low level of protein synthesis observed with the chloroplast RNA in the wheat germ system was due not to the chloroplast RNA but to a small amount of contaminating cytoplasmic RNA. Analysis of the chloroplast RNA on 2.2% polyacrylamide gels revealed the presence of a very small amount of cytoplasmic ribosomal RNA and, although this in itself is not necessarily a measure of the messenger RNA content it is indicative of the possible level of contaminating cytoplasmic mRNA.

Autoradiography of the products from the root RNA-directed wheat germ S30 (fig.2g) revealed the presence of a large number of discrete proteins ranging in size up to 60 000 daltons. We have not attempted to identify any of these products, but, in view of the fact that the wheat germ system is capable of translating many eukaryote mRNAs and viral RNAs, it seemed reasonable to assume that the wheat germ S30 system was also translating root mRNA with fidelity.

As indicated above, the *E. coli* system does respond somewhat to root RNA and the products from this reaction were also examined by SDS-gel electrophoresis. In this case, no discrete bands of radioactivity were observed on the autoradiograph (fig.2h). All the radioactivity migrated as a diffuse area at the gel front indicating that the *E. coli* ribosomes were failing to translate the root mRNA accurately.

If the 70S and 80S S30 cell-free systems are indeed responsive to different sets of messenger RNA then, when presented with a mixture containing both RNAs, each S30 should be able to translate selectively one particular RNA only. A preparation of total leaf RNA potentially represents such a mixture of chloroplast and cytoplasmic messenger RNAs. When the products obtained from reactions involving total leaf RNA and either the *E. coli* S30 or the wheat germ S30 were analysed on SDS-polyacrylamide gels the autoradiograph patterns were found to be quite different (figs.2c,d). Furthermore, the pattern obtained from total leaf RNA and the *E. coli* system was virtually identical to that obtained from chloroplast RNA and the *E. coli* system (cf. figs.2b,c). Likewise, the autoradiograph of the products from the total leaf RNA plus wheat germ system was very similar to that from leaf cytoplasmic RNA plus wheat germ (cf. figs.2d,e). The two ribosomal systems appear to be able to recognize and selectively translate different messenger RNAs present in a mixture of RNAs.

Although the quantitative response of the *E. coli* S30 to leaf cytoplasmic RNA was somewhat less than that to total leaf RNA (data not shown) the pattern of products revealed by polyacrylamide gel electrophoresis was identical in each case and basically the same as that obtained in the chloroplast RNA plus *E. coli* S30 (cf. figs.2a,b,c). This is the result to be expected because the leaf cytoplasmic RNA preparation contained significant amounts of chloroplast RNA.

4. Discussion

The results presented in this paper show that chloroplast mRNA can be differentiated from plant cytoplasmic mRNA. The basis for this conclusion comes from both the quantitative and qualitative response of two types of protein-synthesizing system to the mRNAs in question. Chloroplast mRNA is recognized and translated by a 70S ribosomal system from *E. coli* but not by an 80S ribosomal system from wheat germ. Messenger RNA from plant cytoplasm appears to be recognized and translated by the 80S

ribosomal wheat germ system but not by the *E. coli* system. This highly selective recognition process presumably depends on the presence of specific factors in the two ribosomal systems and on the presence of specific initiation sites in the mRNA from chloroplast and plant cytoplasm. No attempt has been made to analyze this further.

In view of the fact that chloroplasts have a prokaryotic 70S ribosomal protein-synthesizing system and the leaf cytoplasm has an 80S ribosomal system, the possibility that the specific recognition of mRNA types which we have observed using in vitro systems accurately reflects the situation in the leaf cell (and perhaps in other organelle-containing cells) is a very real one. Coding of chloroplast proteins is known to involve both chloroplast and nuclear DNA and the cooperation of both the chloroplast and cytoplasmic protein-synthesizing systems. In the case of the synthesis of the major chloroplast protein, ribulose diphosphate carboxylase, the small subunit is coded by nuclear DNA [10] and translated in the cytoplasm [11] while the large subunit is coded by chloroplast DNA [12] and translated on 70S ribosomes in the chloroplast [13]. If some mRNAs are transcribed in the nucleus but translated in the chloroplast, as suggested by Jennings and Ohad [14], then a protective mechanism must exist to prevent translation during transit from the nucleus to the chloroplast. Again, the existence of a specific recognition process between the ribosomal systems and their homologous mRNAs would be a simple means of achieving this.

Finally, the differential response of the *E. coli* and wheat germ protein-synthesizing systems to chloroplast mRNAs may provide a way of answering the question: 'Are any of the mRNAs which are transcribed from chloroplast DNA ever translated in the cytoplasm?' Chloroplast DNA can be readily transcribed in vitro by *E. coli* RNA polymerase [15]. If RNA transcripts from such a reaction are capable of programming the *E. coli* 70S ribosomal system, but not the wheat germ 80S ribosomal system, then one could conclude that the genetic information encoded in chloroplast DNA is not transcribed but also translated, entirely within the organelle.

References

[1] Boulter, D., Ellis, R. J. and Yarwood, A. (1972) Biol. Rev. 47, 113–175.
[2] Modelell, J. (1971) in: Protein Biosynthesis in Bacterial Systems (Last, J. A. and Laskin, A. I., eds.) pp. 1–65, Dekker, New York.
[3] Johnston, F. B. and Stern, H. (1957) Nature 179, 160–161.
[4] Roberts, B. E. and Paterson, B. M. (1973) Proc. Nat. Acad. Sci. USA 70, 2330–2336.
[5] Bottomley, W., Spencer, D. and Whitfeld, P. R. (1974) Arch. Biochem. Biophys. 164, 106–117.
[6] Spencer, D. and Whitfeld, P. R. (1967) Arch. Biochem. Biophys. 121, 336–345.
[7] Zubay, G., Chambers, D. A. and Cheong, L. C. (1970) in: The Lactose Operon (Beckwith, J. and Zipser, D., eds.) pp. 375–391, Cold Spring Harbor Laboratories, New York.
[8] Laemmli, U. K. and Favre, M. (1973) J. Mol. Biol. 80, 575–599.
[9] Hartley, M. R., Wheeler, A. and Ellis, R. J. (1975) J. Mol. Biol. 91.
[10] Kawashima, N. and Wildman, S. G. (1972) Biochim. biophys. Acta 262, 42–49.
[11] Criddle, R. S., Dau, B., Kleenkopf, G. E. and Huffaker, R. C. (1970) Biochem. Biophys. Res. Comm. 41, 621–627.
[12] Chan, P. and Wildman, S. G. (1972) Biochim. biophys. Acta 227, 677–680.
[13] Blair, G. E. and Ellis, R. J. (1973) Biochim. Biophys. Acta 319, 223.
[14] Jennings, R. C. and Ohad, I. (1972) Arch. Biochem. Biophys. 153, 79–87.
[15] Whitfeld, P. R., Spencer, D. and Bottomley, W. (1973) in: The Biochemistry of Gene Expression in Higher Organisms (Pollak, J. K. and Lee, J. W., eds.) pp. 504–522, Australia & New Zealand Book Co., Sydney.